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(54) **FORMULATIONS COMPRISING JORUMYCIN-, RENIERAMYCIN-, SAFRACIN- OR SAFRAMYCIN-RELATED COMPOUNDS FOR TREATING PROLIFERATIVE DISEASES**

(75) Inventors: **Pilar Calvo Salve**, Madrid (ES); **Maria Tobio Barreira**, Madrid (ES)

(73) Assignee: **Pharma Mar, S.A.**, Madrid (ES)

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See application file for complete search history.

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Primary Examiner — Dennis Heyer
(74) *Attorney, Agent, or Firm* — Kenneth H. Sonnenfeld; Bryte V. Kelly; King & Spalding LLP

(57) **ABSTRACT**

Jorumycin, renieramycin, safracin and saframycin related compounds formulations, methods of preparing the same, articles of manufacture and kits with such formulations, and methods of treating proliferative diseases with the same formulations are provided.

39 Claims, 1 Drawing Sheet

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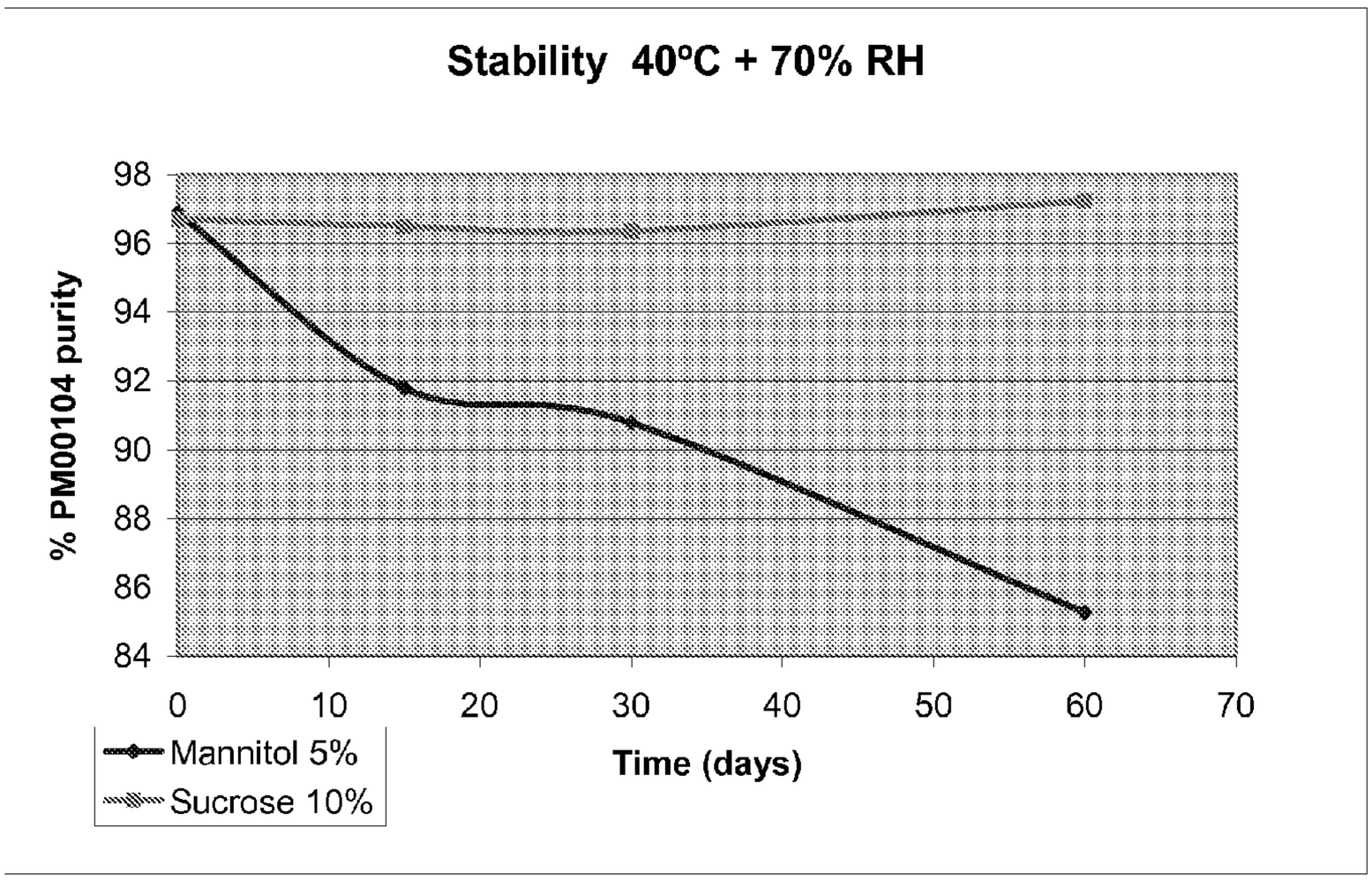

Stability 40ºC + 70% RH
% PM00104 purity
98
96
94
92
90
88
86
84
0
10
20
30
40
50
60
70
Time (days)
Mannitol 5%
Sucrose 10%

FORMULATIONS COMPRISING JORUMYCIN-, RENIERAMYCIN-, SAFRACIN- OR SAFRAMYCIN-RELATED COMPOUNDS FOR TREATING PROLIFERATIVE DISEASES

This application is the national phase entry under 35 U.S.C. §371 of PCT/GB2006/050362, filed Oct. 30, 2006, which claims priority under 35 U.S.C. §119(a)-(d) to GB 0522082.7, filed Oct. 31, 2005, the entire contents of which are hereby incorporated by reference.

The present invention relates to formulations. More particularly it relates to compositions and formulations of jorumycin-, renieramycin-, safracin- and saframycin-related compounds, such as compounds PM00104 and PM00121.

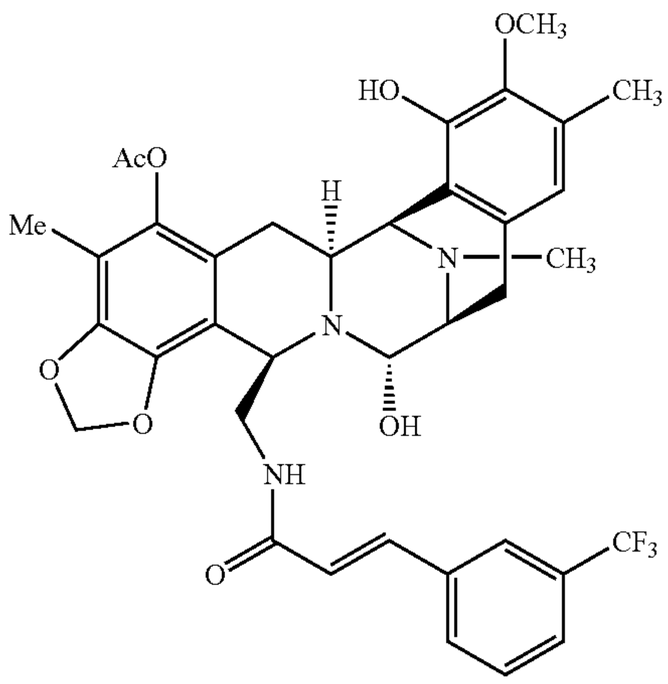
BACKGROUND OF THE INVENTION

Jorumycin is a natural compound isolated from the skin and from the mucus of the Pacific nudibranch *Jorunna funebris* (Fontana A., et al., Tetrahedron (2000), 56, 7305-8). In addition, the family of renieramycins is disclosed as being isolated from sponges and tunicates (James M. F. et al. J. Am. Chem. Soc. (1982), 104, 265-269; Oku N., et al. Journal Natural Products (2003), 66, 1136-9). Safracin and saframycin compounds are disclosed in Manzanares I., et al. Curr. Med. Chem. Anti-Cancer Agents (2001), 1, 257-276, as well as in WO 00/18233 and WO 01/87894.

Because of the detailed description provided in such references and citations therein, the structural characterizations of such compounds are not given again explicitly herein; any person of ordinary skill in this technology is capable of obtaining such information directly from the sources cited here and related sources. At least two of such compounds, PM00104 and PM00121 will be referred to specifically herein to illustrate features of this invention.

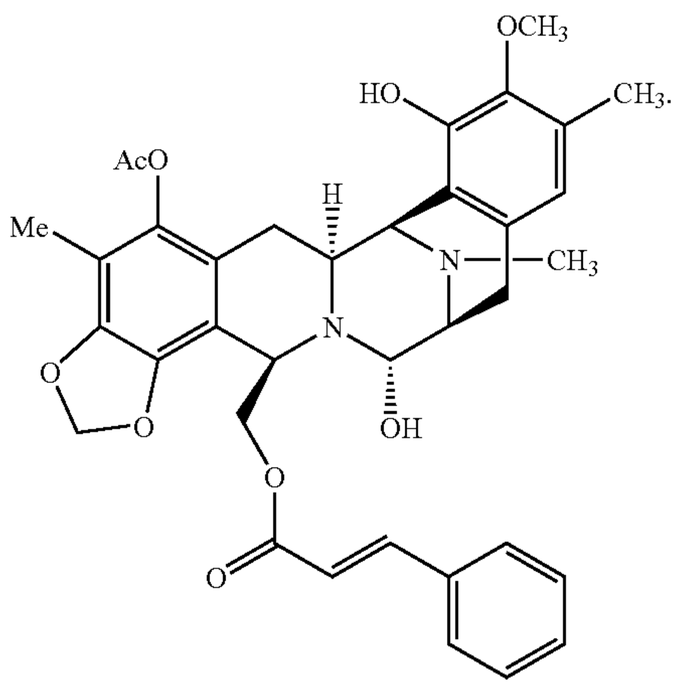
PM00104 and PM00121 are synthetic alkaloids related to jorumycin and renieramycins, and also to safracin and saframycin compounds. They show the following chemical structures:

PM00104



-continued

PM00121



A pharmaceutical composition comprising PM00104 or PM00121 together with a pharmaceutically acceptable carrier is claimed in WO 01/87894.

PM00104 has demonstrated a significant in vitro activity against solid and non-solid tumor cell lines as well as significant in vivo activity in several xenografted human cell lines in mice, such as breast and prostate. Preliminary insights into the mechanism of action of PM00104 suggested cell cycle changes, DNA binding properties and transcriptional inhibition. In addition, clinical phase I studies are currently ongoing with PM00104. For further activity data details of PM00104 and PM00121 see WO 01/87894

PM00104 and PM00121, as well as related compounds, are complex chemical entities, as revealed by their structural features. In addition, they exhibit limited aqueous solubility, and their stability, particularly in biocompatible forms and formulations, is difficult to predict and achieve. These characteristics challenge the ordinary skills and conventional methodologies in this technology, particularly when it comes to the preparation of formulations of these compounds that are to be readily used for medical purposes. Such uses preferably rely on formulations whose characteristics include one or more of the following: biocompatibility, stability under ambient conditions, or under conditions that are as near to ambient conditions as possible, with a shelf life that is as long as possible, and easy reconstitutability to form reconstituted solutions that are as stable under ambient, or near ambient conditions, for as long as possible.

In view of the potential of these compounds as antitumoral agents, there is a need to provide a formulation that can solve problems that conventional formulations and manufacturing methodologies do not address or do not completely solve. These problems include the problem of stability of these compounds. Embodiments of PM00104, PM00121 and related compounds formulations should preferably exhibit favourable freeze-drying properties, should preferably be susceptible of ready reconstitution, and they should preferably exhibit dilution properties, such as upon dilution with infusion fluid, while presenting as many of the desirable characteristics of formulations for medical use as referred to herein. As indicated above, embodiments of these formulations should be stable during long term storage. In addition, the formulation and its manufacturing methodology should satisfy biocompatibility standards and should thus allow for the effective use of a formulation vehicle that is non-toxic, at least at the concentrations used for infusion.

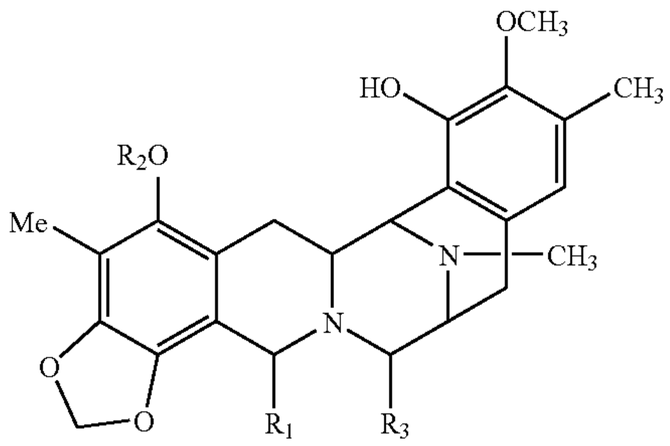
A general review of excipient-drug interactions in parental formulations is provided by Akers M. J., in Journal of Pharmaceutical Sciences, 91, 2002, 2283-2300. This reference provides, inter alia, a section on bulking agents and lyoprotectants, including this subject matter in the context of lyophilisation.

It is envisaged that the methodologies and formulations developed in the context of this invention are applicable to other related compounds, in addition to PM00104 and PM00121.

OBJECTS OF THE INVENTION

Specifically the invention relates to compositions and formulations of compounds of general formula (I):

(I)



wherein R_1 is selected from the group consisting of —CH_2—$N(R_a)_2$ and —CH_2—OR_a, where each R^a is independently selected from the group consisting of H, alkyl-CO—, haloalkyl-CO—, cycloalkylalkyl-CO—, haloalkyl-O—CO—, arylalkyl-CO—, arylalkenyl-CO—, heteroaryl-CO—, alkenyl-CO—, alkyl, alkenyl and amino acid acyl, or the two R_a groups together with the N atom of —CH_2—N$(R_a)_2$ form a heterocyclic group;

R_2 is selected from alkyl-CO—, cycloalkyl-CO— and haloalkyl-CO—; and

R_3 is OH or CN; or

a pharmaceutically acceptable salt, derivative, prodrug or stereoisomer thereof. The various groups can be unsubstituted, or substituted.

Thus, the present invention provides stable formulations of compounds of general formula (I), and methods of making such formulations.

It is an object of this invention to provide a new stable formulation of compounds of general formula (I). In particular, a formulation is needed with great storage stability. In addition, there is especially a need to avoid the formation of impurities.

SUMMARY OF THE INVENTION

According to the present invention there are provided compositions which comprise a compound of general formula (I) and a disaccharide, and methods for preparing such compositions. Preferred embodiments of such compositions are of pharmaceutical purity.

Some embodiments of such compositions are provided by lyophilised formulations which comprise a compound of general formula (I) and a disaccharide. Methods for preparing such formulations are provided.

DETAILS OF THE INVENTION

We have found in the context of this invention that disaccharides stabilise formulations of compounds of general formula (I) as defined above.

In these compounds the substituents can be selected in accordance with the following guidance:

Alkyl groups preferably have from 1 to 12 carbon atoms. One more preferred class of alkyl groups has from 1 to about 6 carbon atoms, and most preferably 1, 2, 3 or 4 carbon atoms. Methyl, ethyl and propyl including isopropyl are particularly preferred alkyl groups in the compounds of the present invention. As used herein, the term alkyl, unless otherwise modified, refers to both cyclic and noncyclic groups, although cyclic groups will comprise at least three carbon ring members.

Preferred alkenyl groups in the compounds of the present invention have one or more unsaturated linkages and from 2 to about 12 carbon atoms. One more preferred class of alkenyl groups has from 2 to about 6 carbon atoms, and most preferably 2, 3 or 4 carbon atoms. The term alkenyl as used herein refers to both cyclic and noncyclic groups.

Suitable aryl groups in the compounds of the present invention include single and multiple ring compounds, including multiple ring compounds that contain separate and/or fused aryl groups. Typical aryl groups contain from 1 to 3 separated or fused rings and from 6 to about 18 carbon ring atoms. Specially preferred aryl groups include substituted or unsubstituted phenyl, naphthyl, biphenyl, phenanthryl and anthracyl.

Suitable heterocyclic groups include heteroaromatic and heteroalicyclic groups. Suitable heteroaromatic groups in the compounds of the present invention contain one, two or three heteroatoms selected from N, O or S atoms and include, e.g., coumarinyl including 8-coumarinyl, quinolinyl including 8-quinolinyl, pyridyl, pyrazinyl, pyrimidyl, furyl, pyrrolyl, thienyl, thiazolyl, oxazolyl, imidazolyl, indolyl, benzofuranyl and benzothiazol groups. Suitable heteroalicyclic groups in the compounds of the present invention contain one, two or three heteroatoms selected from N, O or S atoms and include, e.g., tetrahydrofuranyl, tetrahydropyranyl, piperidinyl, morpholino and pyrrolindinyl groups. Phthalimido is another candidate heterocyclic group.

Suitable amino acid acyl groups include alanyl, arginyl, aspartyl, cystyl, glutamyl, glutaminyl, glycyl, histidyl, hydroxyprolyl, isoleucyl, leucyl, lysyl, methionyl, phenylalanyl, prolyl, seryl, threonyl, tyronyl, tryptophyl, tyrosyl, valyl, as well as other amino acid groups, which may be L- or D-.

The groups mentioned herein may be substituted at one or more available positions by one or more suitable groups such as R', OR', =O, SR', SOR', SO_2R', NO_2, NHR', $N(R')_2$, =N—R', NHCOR', $N(COR')_2$, $NHSO_2R'$, CN, halogen, C(=O)R', CO_2R', OC(=O)R', wherein each of the R' groups is independently selected from hydrogen, OH, NO_2, NH_2, SH, CN, halogen, =O, C(=O)H, C(=O)alkyl, CO_2H, substituted or unsubstituted C_1-C_{12} alkyl, substituted or unsubstituted C_2-C_{12} alkenyl, substituted or unsubstituted C_2-C_{12} alkynyl and substituted or unsubstituted aryl. Suitable halogen substituents in the compounds of the present invention include F, Cl, Br and I. Where such groups are themselves substituted, the substituents may be chosen from R", OR", =O, SR", SOR", $SO_2R"$, NO_2, NHR'",$(R")_2$ =N—R", NHCOR", N $(COR")_2$, $NHSO_2R"$, CN, halogen, C(=O)R", $CO_2R"$, OC(=O)R", wherein each of the R" groups is independently selected from the group consisting of hydrogen, OH, NO_2, NH_2, SH, CN, halogen, =O, C(=O)H, C(=O)

alkyl, CO_2H, unsubstituted C_1-C_{12} alkyl, unsubstituted alkenyl, unsubstituted C_2-C_{12} alkynyl and unsubstituted aryl.

The term "pharmaceutically acceptable salts, derivatives, prodrugs" refers to any pharmaceutically acceptable salt, ester, solvate, hydrate or any other compound which, upon administration to the recipient is capable of providing (directly or indirectly) a compound as described herein. However, it will be appreciated that non-pharmaceutically acceptable salts also fall within the scope of the invention since those may be useful in the preparation of pharmaceutically acceptable salts. The preparation of salts, prodrugs and derivatives can be carried out by methods known in the art.

For instance, pharmaceutically acceptable salts of compounds provided herein are synthesized from the parent compound, which contains a basic or acidic moiety, by conventional chemical methods. Generally, such salts are, for example, prepared by reacting the free acid or base forms of these compounds with a stoichiometric amount of the appropriate base or acid in water or in an organic solvent or in a mixture of the two. Generally, nonaqueous media like ether, ethyl acetate, ethanol, isopropanol or acetonitrile are preferred. Examples of the acid addition salts include mineral acid addition salts such as, for example, hydrochloride, hydrobromide, hydroiodide, sulphate, nitrate, phosphate, and organic acid addition salts such as, for example, acetate, maleate, fumarate, citrate, oxalate, succinate, tartrate, malate, mandelate, methanesulphonate and p-toluenesulphonate. Examples of the alkali addition salts include inorganic salts such as, for example, sodium, potassium, calcium and ammonium salts, and organic alkali salts such as, for example, ethylenediamine, ethanolamine, N,N-dialkylenethanolamine, triethanolamine and basic aminoacids salts.

The compounds of the invention may be in crystalline form either as free compounds or as solvates (e.g. hydrates) and it is intended that both forms are within the scope of the present invention. Methods of salvation are generally known within the art.

Any compound that is a prodrug of a compound of formula (I) is within the scope and spirit of the invention. The term "prodrug" is used in its broadest sense and encompasses those derivatives that are converted in vivo to the compounds of the invention. Such derivatives would readily occur to those skilled in the art, and include, for example, compounds where a free hydroxy group is converted into an ester derivative.

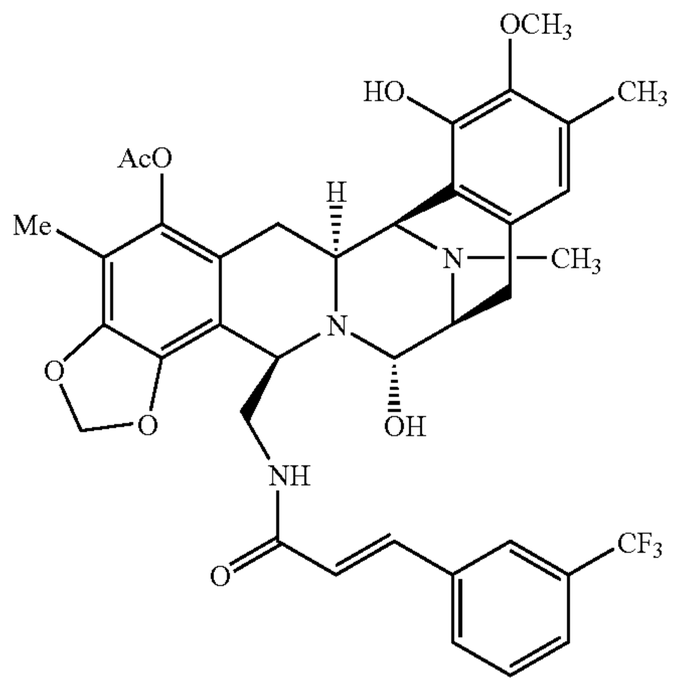
The compounds of the present invention represented by the above described formula (I) may include enantiomers depending on their asymmetry or diastereoisomers. Stereoisomerism about the double bond is also possible, therefore in some cases the molecule could exist as (E)-isomer or (Z)-isomer. The single isomers and mixtures of the isomers fall within the scope of the present invention.

Examples of compounds of the present invention include those disclosed for example in WO 00/18233 and WO 01/87894. We incorporate by specific reference each of the compounds identified in the respective examples of these PCT filings. More generally we incorporate by specific reference the content of these two PCT filings for their disclosure of compounds of present formula (I). We adopt the mention of preferred groups given in those texts, particularly as they apply to the present groups R^1 and R^2, especially R^1.

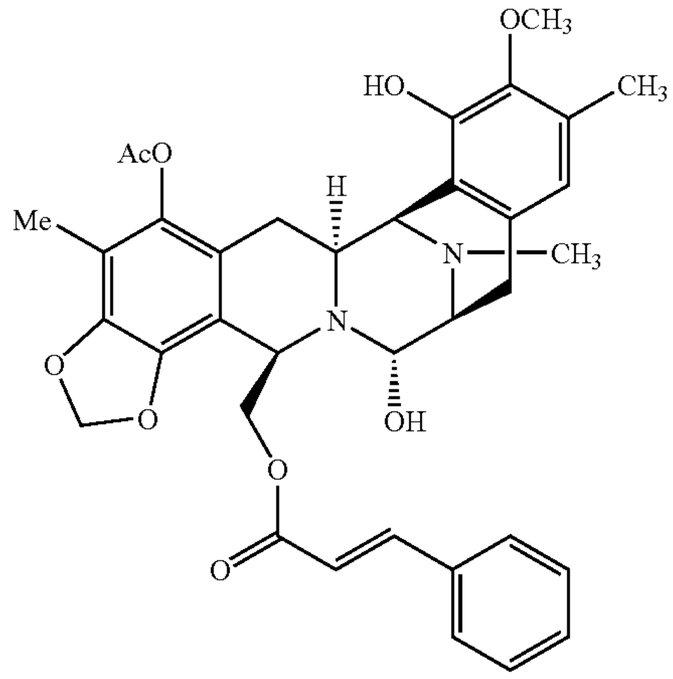
R^3 is usually OH.

Preferred compounds of this invention are those with the following chemical structure:

PM00104



PM00121



Compounds of general formula (I), including PM00104 and PM00121, are complex chemical entities whose behaviour in formulations is not predictable in terms of the behaviour of other unrelated chemical substances. Such behaviour is even more difficult to predict when at least one compound of general formula (I) is included as the active substance in a formulation that is to satisfy biocompatibility standards, including medical standards. We have further found in this regard that the use of disaccharides as bulking agents can drastically reduce the formation of impurities during the lyophilisation process and storage of PM00104 and PM00121 compositions.

In addition, the use of disaccharides also improves the storage conditions allowing long term storage of the lyophilised formulation in a wide temperature range, including refrigeration conditions and room temperature. The term "stable" as used herein in, for example the expression "a stable PM00104 or PM00121 formulation", refers to a formulation that satisfies stability characteristics as reported herein and equivalents thereof, that are not possessed by conventional formulations and that are not achieved when the formulation is prepared by conventional manufacturing methodologies.

Examples of embodiments of the present invention are provided by novel pharmaceutically acceptable compositions comprising a compound of general formula (I) and a disaccharide. Examples of suitable disaccharides include lactose, trehalose, sucrose, and combinations thereof. Additional examples of disaccharides that can be used in some embodiments of this invention include at least one of maltose, iso-

maltose, cellobiose, isosaccharose, isotrehalose, sorbose, turanose, melibiose, gentiobiose, and mixtures thereof. Sucrose is currently preferred. In other embodiments of the invention, the composition comprises a compound of general formula (I) and a lactose free disaccharide. In other embodiments of the invention, the composition comprises a compound of general formula (I) and a trehalose free disaccharide. In other embodiments of the invention, the composition comprises a compound of general formula (I) and a sucrose free disaccharide. In other embodiments of the invention, the composition comprises a compound of general formula (I) and a maltose free disaccharide. In other embodiments of the invention, the composition comprises a compound of general formula (I) and an isomaltose free disaccharide. In other embodiments of the invention, the composition comprises a compound of general formula (I) and a cellobiose free disaccharide. In other embodiments of the invention, the composition comprises a compound of general formula (I) and an isosaccharose free disaccharide. In other embodiments of the invention, the composition comprises a compound of general formula (I) and an isotrehalose free disaccharide. In other embodiments of the invention, the composition comprises a compound of general formula (I) and a sorbose free disaccharide. In other embodiments of the invention, the composition comprises a compound of general formula (I) and a turanose free disaccharide. In other embodiments of the invention, the composition comprises a compound of general formula (I) and a melibiose free disaccharide. In other embodiments of the invention, the composition comprises a compound of general formula (I) and a gentiobiose free disaccharide. Thus, in some embodiments, the composition of this invention contains less than 2% or less than 1% or less than 0.5% or less than 0.2% or less than 0.1% by weight of at least one of, preferably each of, lactose, trehalose, sucrose, maltose, isomaltose, cellobiose, isosaccharose, isotrehalose, sorbose, turanose, melibiose, and gentiobiose.

The terms "mixtures thereof" and "combinations thereof" as used herein refer to at least two entities that provide the antecedent basis for the terms "mixtures thereof" or "combinations thereof". By way of illustration, but not as a limitation, the terms "product comprising at least one of A, B, C, and mixtures thereof" refer to embodiments of the product for which any one of the following is satisfied: A is in the product; B is in the product; C is in the product; A and B are in the product; A and C are in the product; B and C are in the product; and A, B and C are in the product.

Furthermore, it is understood that terms such as "reacting", "forming", and related terms, applied to a chemical entity herein refer to any one of: (a) the chemical entity as such, and (b) the chemical entity in the form in which such entity is present in the reaction medium. Analogously, to name a chemical entity or to give its formula in the context of an operation or reaction step, or to name it or give its formula as being in a medium, whether solid or liquid, including products, formulations, and combinations, refers herein to any one of: (a) the entity as such, and (b) the entity in the form in which such entity is present in the medium. For example, naming an acidic chemical entity herein refers to whichever form or forms such entity is present in the context in which it is named. By way of illustration, but not as a limitation, naming the chemical entity "sodium chloride" or providing its chemical formula refers herein to the entity NaCl as such diatomic molecule, if such is the form in which sodium chloride is present in the relevant medium; it also refers to the collection of undissociated and/or dissociated chemical species if sodium chloride in the relevant medium is entirely or partially dissociated, including species in such medium that are solvated, part of cages, associated with other species, etc.

To provide a more concise description, some of the quantitative expressions given herein are not qualified with the term "about". It is understood that, whether the term "about" is used explicitly or not, every quantity given herein is meant to refer to the actual given value, and it is also meant to refer to the approximation to such given value that would reasonably be inferred based on the ordinary skill in the art, including equivalents and approximations due to the experimental and/or measurement conditions for such given value.

The active substance or substances in the context of this invention can be of natural, semisynthetic or synthetic origin, including combinations of origins. In embodiments where the active substance is a compound such as PM00104 or PM00121, these compounds are of synthetic or semisynthetic origin and can be prepared following the disclosure of WO 01/87894, which is incorporated in full by reference.

The ratio of the active substance to the bulking agent in embodiments of this invention is determined according to the solubility of the bulking agent and, when the formulation is freeze dried, also according to the freeze-dryability of the bulking agent. It is envisaged that this ratio (w/w) can be about 1:1 in some embodiments, about 1:5 in other embodiments, about 1:10 in still other embodiments, while other embodiments illustrate ratios in the range from about 1:10 to about 1:1. It is envisaged that other embodiments have such ratios in the range from about 1:10 to about 1:80, and still further embodiments have such ratios in the range from about 1:80 to about 1:1500. When the active compound is PM00104 or PM00121, the ratio (w/w) of active ingredient to bulking agent is typically from about 1:80 to about 1:1500, preferably from about 1:100 to about 1:800, more preferably from about 1:100 to about 1:400, and even more preferably about 1:200.

The lyophilised material is usually presented in a vial which contains a specified amount of active compound. When the active compound is PM00104, active amounts are illustrated by 2.5 mg/vial. When the active compound is PM00121, active amounts are illustrated by 1 mg/vial.

The present invention is not limited by specific container forms or designs, as long as the container is acceptable for its intended use and standards therefore. Embodiments of this invention are provided with a formulation contained in vials.

The lyophilised formulations of this invention can be reconstituted and diluted to give a composition of this invention in the form of a solution ready for intravenous injection. The actual amounts of reconstituting fluid are not limiting features of embodiments of this invention. By way of illustrations, but not as limitations, embodiments of lyophilised formulations according to this invention are reconstituted with a volume of water. Most of such volumes do not exceed about 20 ml, with preferred volumes being in the range from about 1 ml to about 15 ml, more preferably in the range from about 1 ml to about 10 ml, and even more preferably in the range from about 3 ml to about 8 ml, and even more preferably about 5 ml. When the active substance is embodied by PM00104, the reconstituted solution in such embodiments contains a concentration of PM00104 up to 5 mg/ml, with concentrations of about 2.5 mg/ml, about 1 mg/ml, and about 0.5 mg/ml being preferred.

Reconstituted embodiments of the present invention can further be diluted if so desired, with this further dilution not being a limitation of the present invention. This further dilution is preferably carried out with an aqueous system which is usually 0.9% sodium chloride or 5% glucose. The reconsti-

tuted solution will be diluted depending on the concentration in the reconstituted solution and the desired concentration in the diluted solution.

Embodiments of formulations of compounds of formula (I) according to this invention can be used in the treatment of a variety of cancers. It is understood that "treatment" in this context refers to an action that leads to an amelioration of the cancer condition(s). Furthermore, embodiments of formulations according to this invention can be used in the trials with laboratory tissues, including but not limited to clinical trials, analytical trials, and modelling assays.

Embodiments of this invention that comprise compounds of formula (I) are preferably administered by infusion. The infusing step is typically repeated on a cyclic basis, which may be repeated as appropriate over for instance 1 to 20 cycles. The cycle includes a phase of infusing a formulation of a compound of formula (I), and usually also a phase of not infusing the active substance. Typically the cycle is worked out in weeks, and thus the cycle normally comprises one or more weeks of an active substance infusion phase, and one or more weeks to complete the cycle. We prefer that infusion times of up to 24 hours are used, more preferably 1-12 hours, with 1-6 hours most preferred. Short infusion times which allow treatment to be carried out without an overnight stay in hospital are especially desirable. However, infusion may be 12 to 24 hours or even longer if required.

Embodiments of formulations of this invention that contain a compound of formula (I) can be made by freeze-drying a composition of this invention in the form of a bulk solution including a compound of formula (I) and disaccharide. Usually the bulk solution will be buffered, for example to a pH of about 4. Suitable buffering agents include phosphate buffer, citrate buffer, phosphate/citrate buffer (a mixture of phosphate buffer and citrate buffer), lactate buffer, ascorbate buffer, tartaric/citrate buffer, bicarbonate/hydrochloric acid buffer, acetate buffer, succinate buffer and glycine/hydrochloric acid buffer. Mixtures of buffers can be used. Biocompatible buffers that permit the control of pH at a desired value provide additional embodiments of this invention.

Other components can be included in the bulk solution, for example surface-active agents such as polyoxyethylene sorbitan monooleate (also known as polysorbate) or polyoxyl 40 stearate. Other possible surface-active agents include phospholipids, such as a lecithin; polyoxyethylene-polyoxypropylene copolymers, such as a Pluronic surfactant; polyoxyethylene esters of 12-hydroxysteraric acid, such as a Solutol surfactant; ethoxylates of cholesterol, such as diacyl glycerol, dialkyl glycerol; bile salts, such as sodium cholate, sodium deoxycholate; sucrose esters, such as sucrose monolaurate, sucrose monooleate; polyvinyl pyrrolidone (PVP); or polyvinyl alcohol (PVA).

The formulation is normally supplied as a vial containing the lyophilised product. This supply form, however, is not a limitation of the present invention. To provide a vial containing the lyophilised product, the bulk solution is added to a vial and freeze-dried.

The freeze-drying is carried out in some embodiments of this invention by using reduced secondary drying times. A preferred protocol involves cooling to a temperature of about −40° C. to about −50° C., primary drying at 80 to 85 μbar for 25 to 50 hours, and secondary drying at a lower pressure and at above 0° C. for 3 to 20 hours.

Embodiments of this invention comprise lyophilization by cooling product below −40° C. The primary drying is performed at a temperature from about −20° C. to about −27° C. and a pressure of about 85 μbar for approximately 35 to 46 hours. The secondary drying is carried out at a temperature from about 20° C. to about 25° C. for approximately 30 to 45 hours.

Embodiments of formulations of this invention are suitable for storage at temperatures significantly higher than conventional formulation storage temperatures. Examples of storage temperatures for formulations according to this invention are around +5° C. These temperatures are readily provided by ordinary refrigerators.

DRAWING OF THE INVENTION

FIG. **1**. Comparative PM00104% purity evolution of two PM00104 formulations, one comprising sucrose and the other one mannitol, stored at 40° C./70% RH during 3 months.

EXAMPLES

Example 1

This example discloses a comparative stability study of two PM00104 formulations, one using mannitol as bulking agent, and the other one using sucrose, which is a disaccharide and illustrates the present invention.

The composition of the bulk solution for each of the formulations was as follows (Table I):

TABLE I

Component	Mannitol Formulation	Sucrose Formulation
PM00104	0.1 mg/ml	0.1 mg/ml
Mannitol	5%	—
D-(+)-Sucrose	—	100 mg/ml
Potassium dihydrogen phosphate	6.8 mg/ml	6.8 mg/ml
Phosphoric acid	q.s. to pH 4	q.s. to pH 4
Water for injection	—	q.s. to 1 ml

Bulk solutions were prepared and freeze-dried by a standardised procedure.

Mannitol Formulation

A volume of 50 ml of mannitol formulation was prepared: 40 ml of a solution of potassium dihydrogen phosphate 0.05M (pH 4) was added to 5.493 mg of PM00104, and the mixture was maintained in agitation for 1 hour.

Then, 2.5 g of mannitol was added, washing the plate with 5 ml of a solution of phosphate buffer (pH 4). The mixture was stirred for one hour more. Following, the pH of the solution was adjusted to pH 4 with 1N phosphoric acid and the solution was brought to final weight of 52 g with phosphate buffer 0.05M (pH 4).

The solution was filtered through a PVDF filter and the filtered solution was filled into 10 ml glass vials at 2 ml/vial and vials were lyophilised according to the following procedure (Table II):

TABLE II

Cycle	Step	Pressure	Setpoint T (° C.)	Slope (min)	Holding time
Loading	Shelves T[a]		5° C.		
Freezing	Freeze 1		−45° C.	0.5° C./min	2 h 10 min
	Freeze 2		−45° C.		4 h
Vacuum	Ch vacuum	0.5 mb			

TABLE II-continued

Cycle	Step	Pressure	Setpoint T (° C.)	Slope (min)	Holding time
Sublimation	1° drying	0.150 mb	−23° C.	0.5° C./min	2 h 30 min
	1° drying	0.150 mb	−23° C.		30 h
2nd drying	2° drying		20° C.	0.25° C./min	3 h 30 min
	2° drying		20° C.		6 h
	2° drying		25° C.		30 min
	2° drying		25° C.		40 h

After freeze-drying, the vials were sealed and were transferred to a refrigerated area (−20° C.).

Sucrose Formulation

A volume of 300 ml of sucrose formulation was prepared:

32.615 mg of PM00104 was added to 100 ml of a solution of potassium dihydrogen phosphate 0.05M (pH 4), washing the plate with additional 110 ml of the solution of potassium dihydrogen phosphate 0.05M (pH 4). Then, the mixture was maintained in agitation for 1 hour.

30 g of sucrose was added, washing the plate with 30 ml of solution of phosphate buffer (pH 4). The mixture was maintained in agitation for one hour more.

Following, the pH of the solution was adjusted to pH 4 with 1M phosphoric acid and the solution was brought to final weight of 300 g with water for injection.

The solution was filtered through a Millipore-Optiscale filter and the filtered solution was filled into 10 ml glass vials at 2 ml/vial and vials were lyophilised.

Stability testing was carried at a temperature of 5° C., 25° C./60% RH and 40° C./75% RH in the case of sucrose formulation and 40° C./75% RH in the case of mannitol formulation.

Table III and FIG. **1** show PM00104 chromatographic purity evolution of the formulations under study:

TABLE III

	PM00104 purity (%)			
	5° C.	25° C./60% RH	40° C./75% RH	
	Sucrose Formulation	Sucrose Formulation	Sucrose Formulation	Mannitol Formulation
t = 0	96.70%	96.70%	96.70%	96.89%
t = 15 days	96.85%	96.44%	96.50%	91.79%
t = 1 month	96.74%	96.71%	96.36%	90.79%
t = 2 months	97.23%	97.10%	97.24%	85.26%

Data in table III and FIG. **1** show that formulation containing sucrose displayed an improved stability at 40° C. and 75% RH with an insignificant purity decrease. This decrease is significantly lower than the decrease observed with the mannitol formulation.

Example 2

A PM00121 formulation comprising sucrose as bulking agent was prepared and its stability was evaluated at a temperature of 5° C., 25° C./60% RH and 40° C./75% RH.

For each vial the composition of the bulk solution was as follows (Table IV):

TABLE IV

Component	mg/vial
PM00121	1 mg
Sucrose	200 mg
Potassium dihydrogen phosphate	13.6 mg
Polysorbate 80	0.2 mg
Phosphoric acid	q.s. to pH 4
Water for injection	q.s. to 2 ml

PM00121 formulation was prepared as follows:

100 ml of a solution of polysorbate 80 0.1% (pH 2.5) was added to 161.05 mg of PM00121, and subsequently additional 110 ml of solution of polysorbate 80 0.1% (pH 2.5) was also added. The mixture is maintained in agitation for 1 hour.

Then, 2.04 g of potassium dihydrogen phosphate was added, washing the plate with 15 ml of solution of polysorbate 80 0.1% (pH 2.5).

Following, 30 g of sucrose was weighed and added to the solution, washing the plate with 15 ml of solution of polysorbate 80 0.1% (pH 2.5). Then, the mixture is maintained in agitation for more than 1 hour.

Following, the pH of the solution was adjusted to pH 4 with 1M phosphoric acid and the solution was brought to final weight of 300 g with water for injection.

The solution was filtered with a Millipore-Optiscale filter. The filtered solution was filled into 10 ml glass vials at 2 ml/vial and maintained at −20° C. until the lyophilization process.

Lyophilization process was performed according to the following table V:

TABLE V

Freezing time to −45° C.:	150 min
Primary drying at 115 mTorr and −20° C.	2300 min
Secondary drying at 75 mTorr and 25° C.	600 min

After freeze-drying, the vials were sealed and transferred to a refrigerated area (−20° C.).

Stability testing was carried at a temperature of 5° C., 25° C./60% RH and 40° C./75% RH.

Table VI discloses the PM00121 chromatographic purity of the formulation under study:

TABLE VI

	PM00121 purity (%)		
	5° C.	25° C./60% RH	40° C./75% RH
t = 0	96.92%	96.92%	96.92%
t = 15 days	97.83%	97.63%	97.38%
t = 1 month	97.96%	97.85%	97.46%
t = 2 months	97.98%	97.12%	95.71%

It was noted that the formulation comprising the disaccharide was stable at 5° C. and 25° C./60% RH.

Example 3

Two PM00104 formulations, 104-F A and 104-F B, comprising sucrose as bulking agent were prepared and its stability was evaluated at a temperature of −20° C., 5° C., 25° C./60% RH and 45° C./75% RH.

For each formulation the composition of the bulk solution for each vial was as follows (Table VII):

TABLE VII

Component	mg/vial
PM00104	2.5 mg
D-(+)-Sucrose	500 mg
Potassium dihydrogen phosphate	34 mg
Phosphoric acid	q.s to pH 4
Water for injection	q.s to 5 ml

Bulk solutions were prepared and freeze-dried using the following particular protocols:

Formulation 104-F A

1.750 l of bulk solution was prepared as follows:

153.125 ml of phosphoric acid 0.05N was added to 905.61 mg of PM00104. The mixture was stirred for 15 minutes. Then 1400 ml of water for injection was added, followed by the addition of 11.9 g of potassium dihydrogen phosphate and 175 g of sucrose. The mixture was maintained again in agitation for 1 h 15 min.

The pH of the solution was not needed to be adjusted to 3.8≤pH≤4, since its pH value was 3.91. The solution was brought to final weight of 1820 g with water for injection.

Then, the solution was filtered through a 0.22 μm Millipack®-20 filter. And the filtered solution was filled into 25 ml vials at 5.4 ml of bulk solution/vial and maintained at −20° C. until the lyophilization process.

Lyophilization process was performed according to the following table VIII:

TABLE VIII

Cycle	Step	Pressure	Setpoint T (° C.)	Slope (min)	Holding time
Loading	Shelves T[a]		−5° C.		10 min
Freezing	Freeze 1		−50° C.	0.5° C./min	1 h 50 min
	Freeze 2		−50° C.		3 h
Vacuum	Ch vacuum	0.5 mb			
Sublimation	1° drying	0.080 mb	−27° C.	0.5° C./min	45 min
	1° drying	0.080 mb	−27° C.		45 h
2nd drying	2° drying		25° C.	0.25° C./min	3 h 30 min
	2° drying		25° C.		40 h
	stoppering		25° C.		

The vials were sealed and transferred to a refrigerated area (−20° C.).

Formulation 104-F B

2.271 g of PM00104 was added to 100 ml of phosphoric acid 0.05N, washing the plate with 265 ml of phosphoric acid 0.05N. The mixture was stirred for 15 minutes. Then 3360 ml of water for injection was added, followed by the addition of 28.56 g of potassium dihydrogen phosphate. The mixture was stirred for 3 minutes and 420 g of sucrose was added. The mixture was maintained again in agitation for 1 h 15 min.

The pH of the solution was not needed to be adjusted to 3.8≤pH≤4, since its pH value was 3.84. The solution was brought to final weight of 4369 g with water for injection.

Then, the solution was filtered through a 0.22 μm filter. And the filtered solution was filled into 25 ml vials at 5 ml of bulk solution/vial and maintained at −20° C. until the lyophilization process.

Lyophilization process was performed according to the following table IX:

TABLE IX

Cycle	Step	Pressure	Setpoint T (° C.)	Slope (min)	Holding time
Loading	Shelves T[a]		−5° C.		80 min
Freezing	Freeze 1		−40° C.	0.5° C./min	1 h 50 min
	Freeze 2		−40° C.		3 h
Vacuum	Ch vacuum	0.4 mb			
Sublimation	1° drying	0.085 mb	−27° C.	0.5° C./min	45 min
	1° drying	0.085 mb	−27° C.		35 h
2nd drying	2° drying		25° C.	0.25° C./min	3 h 30 min
	2° drying		25° C.		30 h
	stoppering		25° C.		

After freeze-drying, the vials were sealed and were transferred to a refrigerated area (−20° C.).

Stability testing was carried with both formulations at a temperature of −20° C.±5° C., 5° C.±3° C., 25° C.±2° C./60% RH±5% RH and 40° C.±2° C./75% RH±5% RH.

Table X shows the PM00104 chromatographic purity evolution of the formulation 104-FA during storage at −20° C., 5° C., 25° C./60% RH and 40° C./70% RH.

TABLE X

	PM00104 purity (%)			
	−20° C.	5° C.	25° C./60% RH	40° C./75% RH
t = 0	98.24%	98.24%	98.24%	98.24%
t = 1 month	—	—	—	98.13%
t = 2 months	—	—	98.26%	98.16%
t = 3 months	—	97.97%	98.11%	97.98%
t = 6 months	98.13%	98.09%	98.09%	—
t = 9 months	—	98.07%	—	—
t = 12 months	98.02%	97.99%	—	—

Table XI shows the PM00104 chromatographic purity evolution of the formulation 104-FB during storage at −20° C., 5° C., 25° C./60% RH and 40° C./70% RH.

TABLE XI

	PM00104 purity (%)			
	−20° C.	5° C.	25° C./60% RH	40° C./75% RH
t = 0	98.54%	98.54%	98.54%	98.54%
t = 1 month	—	—	—	98.20%
t = 2 months	—	—	—	98.01%
t = 3 months	—	98.44%	98.37%	97.78%
t = 6 months	98.37%	98.33%	98.25%	96.78%
t = 9 months	—	98.19%	98.15%	—
t = 12 months	97.91%	97.91%	97.55%	—
t = 18 months	98.22%	98.15%	98.12%	—
t = 24 months	98.32%	98.29%	97.77%	—

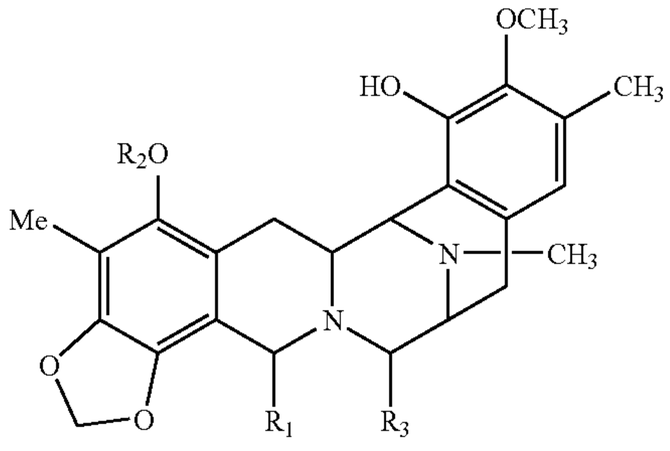
Data in tables X and XI show that the purity evolution of formulations stored 5° C. and 25° C./60% RH is comparable to those of formulation stored at −20° C. Therefore no major degradation is found at 5° C. and 25° C./60% RH showing that formulations comprising a disaccharide can be in storage at least at +5° C. during a long period of time.

All the references cited herein are incorporated by reference in their entirety. The features and advantages of this invention are apparent in light of the disclosure provided herein. Based on this disclosure, modifications and adaptations to various conditions and usages can be made, thus generating embodiments within the scope of this invention.

The invention claimed is:

1. A pharmaceutical composition comprising a disaccharide and a compound of general formula (I):

(I)



wherein R_1 is selected from the group consisting of $—CH_2—N(R^a)_2$ and $—CH_2—OR^a$, where each R^a is independently selected from the group consisting of H, alkyl-CO—, haloalkyl-CO—, cycloalkylalkyl-CO—, haloalkyl-O—CO—, arylalkyl-CO—, arylalkenyl-CO—, heteroaryl-CO—, alkenyl-CO—, alkyl, alkenyl and amino acid acyl, or the two R^a groups together with the N atom of $—CH_2—N(R^a)_2$ form a heterocyclic group;

R_2 is selected from alkyl-CO—, cycloalkyl-CO— and haloalkyl-CO—; and

R_3 is OH or CN; or

a pharmaceutically acceptable salt or stereoisomer thereof; and

wherein said R_1 and R_2 independently, are unsubstituted or substituted at one or more available positions by one or more groups selected from R', OR', =O, SR', SOR', SO_2R', NO_2, NHR', $N(R')_2$, =N—R', NHCOR', $N(COR')_2$, $NHSO_2R'$, CN, halogen, C(=O)R', CO_2R', OC(=O)R',

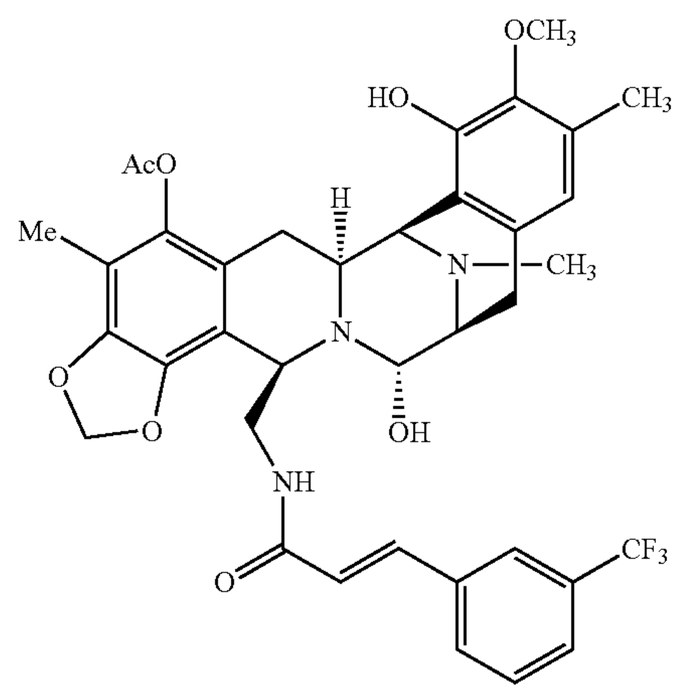
wherein each of the R' groups is independently selected from the group consisting of hydrogen, OH, NO_2, NH_2, SH, CN, halogen, =O, C(=O)H, C(=O)alkyl, CO_2H, substituted or unsubstituted C_1-C_{12} alkyl, substituted or unsubstituted C_2-C_{12} alkenyl, substituted or unsubstituted C_2-C_{12} alkynyl and substituted or unsubstituted aryl,

where, when such R' groups are themselves substituted, the substituents of R' are independently selected from the group consisting of R", OR", =O, SR", SOR", $SO_2R"$, NO_2, NHR", $N(R")_2$, =N—R", NHCOR", $N(COR")_2$, $NHSO_2R"$, CN, halogen, C(=O)R", $CO_2R"$, OC(=O)R",

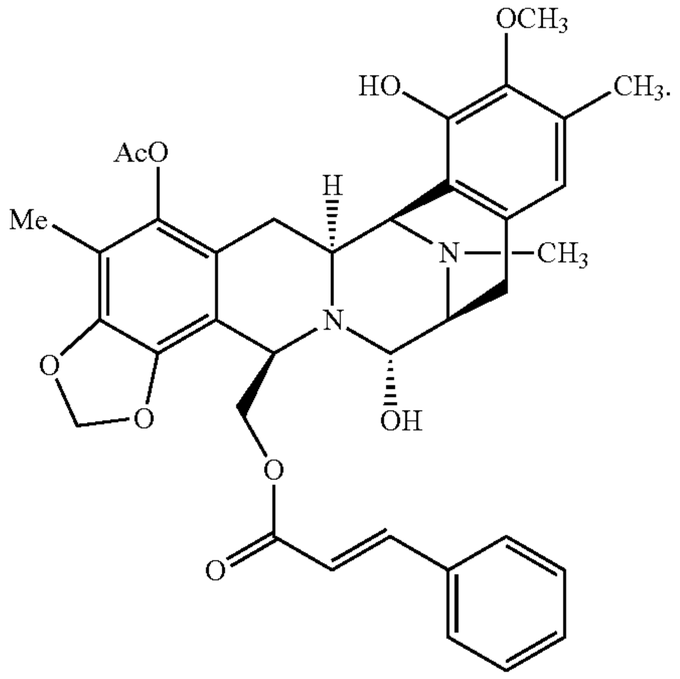
wherein each R" groups is independently selected from the group consisting of hydrogen, OH, NO_2, NH_2, SH, CN, halogen, =O, C(=O)H, C(=O)alkyl, CO_2H, unsubstituted C_1-C_{12} alkyl, unsubstituted C_2-C_{12} alkenyl, unsubstituted C_2-C_{12} alkynyl and unsubstituted aryl.

2. A composition according to claim **1**, wherein said compound is the compound of formula (II) (PM00104) or formula (III) (PM00121):

(II)



(III)



3. A composition according to claim **1**, wherein said disaccharide is selected from the group consisting of lactose, trehalose, sucrose, and mixtures thereof.

4. A composition according to claim **3**, wherein said disaccharide is sucrose.

5. A composition according to claim **1**, wherein the ratio (w/w) of compound to disaccharide is from about 1:80 to about 1:1500.

6. A composition according to claim **5**, wherein the ratio (w/w) of compound to disaccharide is from about 1:100 to about 1:400.

7. A composition according to claim **6**, wherein the ratio (w/w) of compound to disaccharide is about 1:200.

8. A composition according to claim **1**, which further comprises a buffering agent.

9. A composition according to claim **8**, wherein said buffering agent is a phosphate buffer.

10. A composition according to claim **1** which further comprises a surface-active agent.

11. A composition according to claim **10**, wherein the surface-active agent is a polyoxyethylene sorbitan monooleate.

12. A composition according to claim **1**, wherein the composition is in the form of a lyophilised formulation.

13. A composition according to claim **1**, wherein the composition is in the form of a lyophilised formulation.

14. A composition according to claim **13**, wherein said PM00104 is in present in said composition an amount of about 2.5 mg.

15. A composition according to claim **14**, wherein said disaccharide is sucrose, wherein said sucrose is present in said composition in an amount of about 500 mg and wherein said formulation further comprises about 34 mg phosphate, wherein said 34 mg phosphate is calculated as potassium dihydrogen phosphate.

16. A composition according to claim **3**, wherein said disaccharide is selected from the group consisting of lactose, trehalose, sucrose, and mixtures thereof.

17. A composition according to claim **16**, wherein said disaccharide is sucrose.

18. A composition according to claim **3**, wherein the ratio (w/w) of compound to disaccharide is from about 1:80 to about 1:1500.

19. A composition according to claim **18**, wherein the ratio (w/w) of compound to disaccharide is from about 1:100 to about 1:400.

20. A composition according to claim **19**, wherein the ratio (w/w) of compound to disaccharide is about 1:200.

21. A composition according to claim **3**, which further comprises a buffering agent.

22. A composition according to claim **21**, wherein said buffering agent is a phosphate buffer.

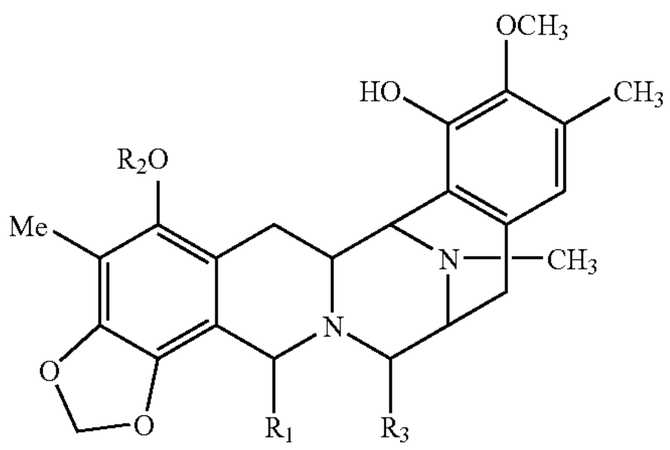
23. A composition according to claim **3** which further comprises a surface-active agent.

24. A composition according to claim **23**, wherein the surface-active agent is a polyoxyethylene sorbitan monooleate.

25. A composition according to claim **3**, wherein said composition does not include a surface-active agent.

26. A pharmaceutical composition comprising a disaccharide and a compound of general formula (I):

(I)



wherein R_1 is selected from the group consisting of $—CH_2—N(R^a)_2$ and $—CH_2—OR^a$, where each R^a is independently selected from the group consisting of H, alkyl-CO—, haloalkyl-CO—, cycloalkylalkyl-CO—, haloalkyl-O—CO—, arylalkyl-CO—, arylalkenyl-CO—, heteroaryl-CO—, alkenyl-CO—, alkyl, alkenyl and amino acid acyl, or the two R^a groups together with the N atom of $—CH_2—N(R^a)_2$ form a heterocyclic group;

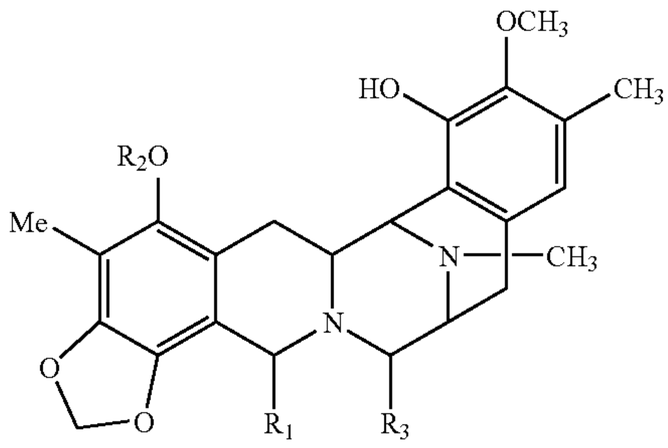
R_2 is selected from alkyl-CO—, cycloalkyl-CO—and haloalkyl-CO—; and

R_3 is OH or CN; or

a pharmaceutically acceptable salt or stereoisomer thereof.

27. A pharmaceutical composition comprising a disaccharide and a single active anti-tumor agent selected from compounds of general formula (I):

(I)



wherein R_1 is selected from the group consisting of $—CH_2—N(R^a)_2$ and $—CH_2—OR^a$, where each R^a is independently selected from the group consisting of H, alkyl-CO—, haloalkyl-CO—, cycloalkylalkyl-CO—, haloalkyl-O—CO—, arylalkyl-CO—, arylalkenyl-CO—, heteroaryl-CO—, alkenyl-CO—, alkyl, alkenyl and amino acid acyl, or the two R^a groups together with the N atom of $—CH_2—N(R^a)_2$ form a heterocyclic group;

R_2 is selected from alkyl-CO—, cycloalkyl-CO— and haloalkyl-CO—; and

R_3 is OH or CN; or

a pharmaceutically acceptable salt or stereoisomer thereof; and

wherein said R_1 and R_2 independently, are unsubstituted or substituted at one or more available positions by one or more groups selected from R', OR', =O, SR', SOR', SO_2R', NO_2, NHR', $N(R')_2$, =N—R', NHCOR', $N(COR')_2$, $NHSO_2R'$, CN, halogen, C(=O)R', CO_2R', OC(=O)R',

wherein each of the R' groups is independently selected from the group consisting of hydrogen, OH, NO_2, NH_2, SH, CN, halogen, =O, C(=O)H, C(=O)alkyl, CO_2H, substituted or unsubstituted C_1-C_{12} alkyl, substituted or unsubstituted C_2-C_{12} alkenyl, substituted or unsubstituted C_2-C_{12} alkynyl and substituted or unsubstituted aryl,

where, when such R' groups are themselves substituted, the substituents of R' are independently selected from the group consisting of R", OR", =O, SR", SOR", $SO_2R"$, NO_2, NHR", $N(R")_2$, =N—R", NHCOR", $N(COR")_2$, $NHSO_2R"$, CN, halogen, C(=O)R", $CO_2R"$, OC(=O)R",

wherein each R" groups is independently selected from the group consisting of hydrogen, OH, NO_2, NH_2, SH, CN, halogen, =O, C(=O)H, C(=O)alkyl, CO_2H, unsubstituted C_1-C_{12} alkyl, unsubstituted C_2-C_{12} alkenyl, unsubstituted C_2-C_{12} alkynyl and unsubstituted aryl.

28. The composition according to claim **1**, wherein said composition has improved stability compared to a formulation using mannitol instead of the disaccharide.

29. The composition according to claim **2**, wherein said composition has improved stability compared to a formulation using mannitol instead of the disaccharide.

30. The composition according to claim **1**, wherein said disaccharide is present in an amount sufficient to inhibit degradation of the anti-tumor agent after storage at 5° C. for 1

month compared to the same composition comprising mannitol instead of the disaccharide.

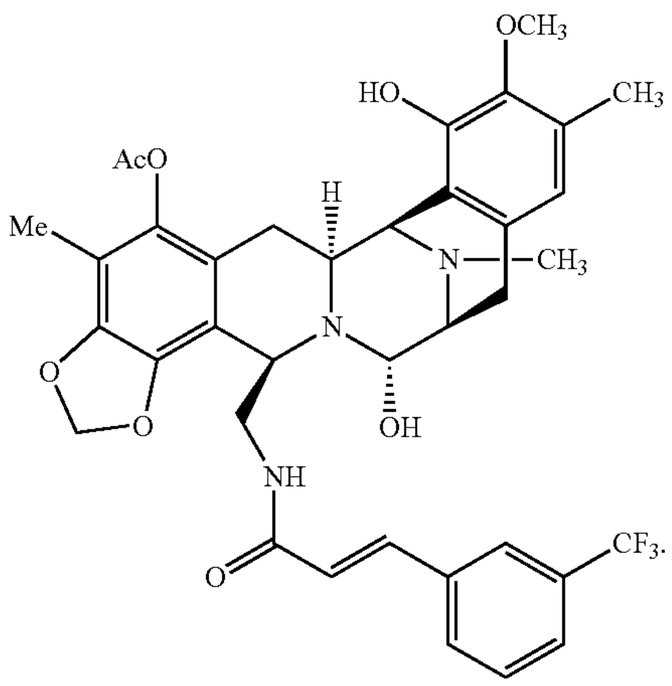
31. The composition according to claim **1**, wherein said disaccharide is present in an amount sufficient to inhibit degradation of the anti-tumor agent after storage at 25° C. for 1 month compared to a composition comprising mannitol instead of the disaccharide.

32. The composition according to claim **1**, wherein said disaccharide is present in an amount sufficient to inhibit degradation of the anti-tumor agent after storage of at 40° C. for 1 month compared to a composition comprising mannitol instead of the disaccharide.

33. A method of making a lyophilised formulation according to claim **12**, comprising freeze-drying a bulk solution that comprises said compound and said disaccharide.

34. A method according to claim **33**, wherein the compound is the compound of formula (II) (PM00104):

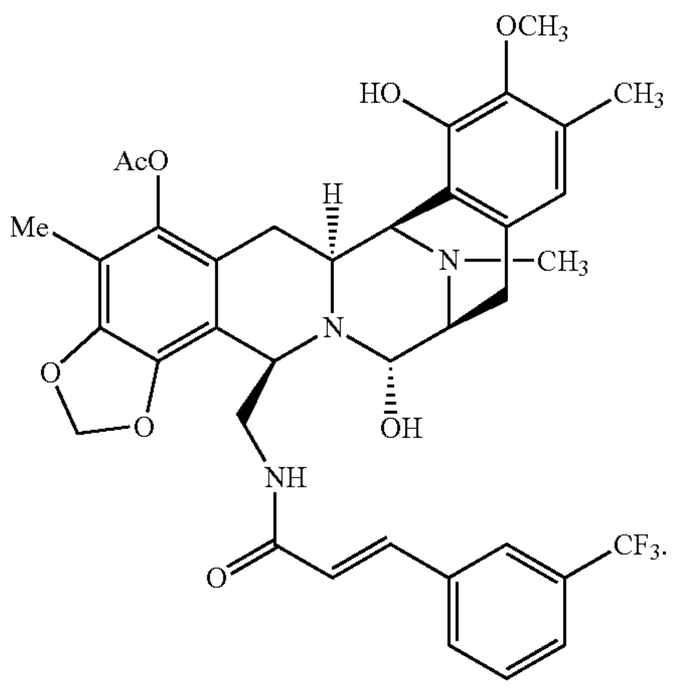
(II)



35. A method of reducing the formation of impurities in said lyophilised formulation according to claim **12**, comprising freeze-drying a bulk solution that comprises said compound and said disaccharide.

36. A method according to claim **35**, wherein the compound is the compound of formula (II) (PM00104):

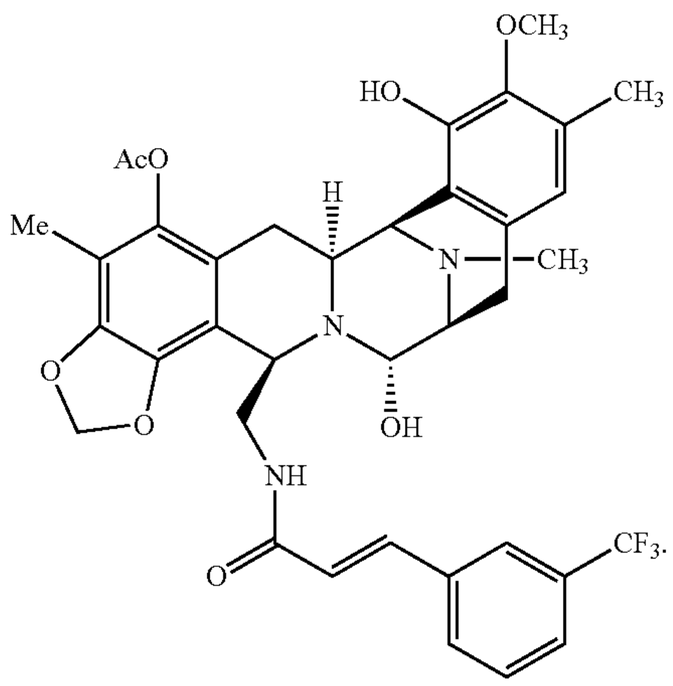
(II)



37. A method of preparing a solution for intravenous infusion, comprising: providing a lyophilised formulation according to claim **12**, adding water to form a reconstituted solution, and diluting said reconstituted solution with an aqueous system.

38. A method according to claim **37**, wherein the compound is the compound of formula (II) (PM00104):

(II)



39. A method of treating cancer, which comprises intravenous infusion of a solution prepared by a method according to claim **37** or **38**.

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